Atty. Docket No.: 28079/41785 Reply to Office Action of 2008 November 25

## **AMENDMENT TO THE CLAIMS**

Amendments to the claims are reflected in the following listing of claims, which replaces all prior versions or listing of claims.

1-46. (Canceled)

- 47. (Currently Amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) starting with a coding sequence, derived from *Bacillus thuringiensis* (B.t.), that encodes an insecticidal protein polypeptide and that contains a plurality of sequences selected from the group consisting of ATTTA sequences and polyadenylation signal sequences listed in Table II;
- (b) reducing the number of said ATTTA sequences or the number of said polyadenylation signal sequences in the coding sequence by substituting sense codons for codons in the coding sequence; and
- (c) making a structural gene that comprises a coding sequence that includes the codons substituted according to step (b) and is characterized by the reduced number of ATTTA sequences or Table II polyadenylation signal sequences, and that encodes the [[an]] insecticidal protein.
  - 48. (Canceled)
- 49. (Currently Amended) The method of claim 47, wherein the coding sequence derived from B.t. contains ATTTA sequences, wherein step (b) the method further comprises reducing the number of said ATTTA sequences in the coding sequence by substituting sense codons for codons in the coding sequence, and wherein the structural gene of step (c) is characterized by the reduced number of ATTTA sequences.
- (Currently Amended) The method of claim [[47,]]49, wherein step (b) 50. comprises reducing the number of saidthe structural gene of step (c) is devoid or substantially devoid of ATTTA sequences and the number of said or devoid or substantially devoid of polyadenylation signal sequences listed in Table II in the coding sequence.

51. (Currently Amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:

- (a) starting with a portion of a coding sequence, wherein the coding sequence is derived from *Bacillus thuringiensis* (*B.t.*), that and encodes an insecticidal protein polypeptide and that wherein the portion contains a plurality of sequences selected from the group consisting of ATTTA sequences and polyadenylation signal sequences listed in Table II;
- (b) reducing the number of said ATTTA sequences [[or]] <u>and</u> the number of said polyadenylation signal sequences in [[a]] <u>said</u> portion of the coding sequence by substituting sense codons for codons in said portion; and
- (c) making a structural gene that comprises said portion with the substitute codons and the reduced number of ATTTA [[or]] and polyadenylation signal sequences, wherein the structural gene comprises a nucleotide sequence that encodes an insecticidal protein.
  - 52. (Canceled)
- 53. (Currently Amended) The method of claim 51, wherein step (b) eomprises reducing the number of said the structural gene of step (c) is devoid or substantially devoid of polyadenylation signal sequences in said portion listed in Table II, or is devoid or substantially devoid of ATTTA sequences.
- 54. (Currently Amended) The method of claim 51, wherein step (b) comprises reducing the number of said ATTTA sequences and the number of said the structural gene of step (c) is devoid or substantially devoid of polyadenylation signal sequences in said portion listed in Table II, and is devoid or substantially devoid of ATTTA sequences.
- 55. (Currently amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) starting with <u>a</u> coding sequence[[s]], from one or more structural genes derived from *Bacillus thuringiensis* (*B.t.*), that encodes an amino acid sequence an insecticidal polypeptide and that contains a plurality of sequences selected from the group consisting of ATTTA sequences and polyadenylation signal sequences listed in Table II;

Application No. 08/434,105 Atty. Docket No.: 28079/41785

Reply to Office Action of 2008 November 25

(b) reducing the number of said ATTTA sequences [[or]] <u>and</u> the number of said polyadenylation signal sequences in <u>a portion of</u> the coding sequence[[s]] by substituting sense codons for codons in <u>said portion</u>, <u>while maintaining the amino acid sequence</u> the <u>eoding sequences</u>; and

- (c) making a structural gene that comprises <u>said portion</u> the coding sequences with the codons substituted according to step (b) and characterized by the reduced number of ATTTA [[or]] <u>and</u> polyadenylation signal sequences, wherein the structural gene comprises a nucleotide sequence that encodes an insecticidal protein.
- 56. (Currently amended) The method of claim 55, wherein the structural gene of step (c) is devoid or substantially devoid of polyadenylation signal sequences listed in Table II, or is devoid or substantially devoid of ATTTA sequences step (b) comprises reducing the number of said ATTTA sequences in said coding sequences.
- 57. (Currently amended) The method of claim 55, wherein the structural gene of step (c) is devoid or substantially devoid of polyadenylation signal sequences listed in Table II, and is devoid or substantially devoid of ATTTA sequences step (b) comprises reducing the number of said polyadenylation signal sequences in said coding sequences.
  - 58. (Canceled)
- 59. (Currently Amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) starting with an amino acid sequence of an insecticidal protein derived from *Bacillus thuringiensis* (*B.t.*), wherein wild-type *B.t.* gene sequence(s) encoding insecticidal polypeptide(s) from which the insecticidal protein is derived comprise a plurality of nucleotide sequences selected from the group consisting of ATTTA sequence(s) and polyadenylation signal sequences listed in Table II; and
  - (b) making a structural gene that comprises a coding sequence that:
    - (i) encodes the amino acid sequence of the insecticidal protein; and
- (ii) contains fewer polyadenylation signal sequences listed in Table II or fewer ATTTA sequences, compared to the corresponding coding sequence(s) of the wild-type <u>B.t.</u> <u>Bacillus</u> gene sequences(s).

60. (Currently Amended) The method of claim 59, wherein the wild-type <u>B.t. Bacillus</u> gene sequence(s) <u>further</u> comprise a <u>plurality of ATTTA</u> sequences, and wherein the structural gene made according to step (b) contains fewer ATTTA sequences compared to the wild-type <u>Bacillus B.t.</u> gene sequences(s).

- 61. (Currently Amended) The method of claim 59, wherein the wild type Bacillus gene sequence(s) comprise a plurality of said polyadenylation signal sequences, and 60, wherein the structural gene made according to step (b) contains fewer is devoid or substantially devoid of said polyadenylation signal sequences or is devoid or substantially devoid of said ATTTA sequences compared to the wild type Bacillus gene sequence(s).
- 62. (Currently Amended) The method of claim 59, wherein the wild type *Bacillus* gene sequence(s) comprise a plurality of both said ATTTA and said polyadenylation signal sequences, and 60, wherein the structural gene made according to step (b) contains fewer is devoid or substantially devoid of ATTTA sequences and is devoid or substantially devoid of said fewer polyadenylation signal sequences compared to the wild type *Bacillus* gene sequence(s).
- 63. (Currently Amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) starting with an amino acid sequence of an insecticidal protein derived from *Bacillus thuringiensis* (*B.t.*); and
- (b) making a structural gene that comprises a coding sequence that encodes the amino acid sequence and that is devoid or substantially devoid of ATTTA sequences, or devoid or substantially devoid of polyadenylation signal sequences listed in Table II.
- 64. (Currently amended) The method of claim 63, wherein step (b) comprises making a structural gene that <u>also</u> is devoid or substantially devoid of the ATTTA sequences.
- 65. (Currently amended) The method of claim 63, wherein step (b) comprises making a structural gene that comprises a coding sequence that is devoid or substantially devoid of the polyadenylation signal sequences.
- 66. (Currently amended) The method of claim [[63]]64, wherein step (b) comprises making a structural gene that comprises a coding sequence that is devoid or

Reply to Office Action of 2008 November 25

substantially devoid of the ATTTA sequences and devoid or substantially devoid of the polyadenylation signal sequences.

- 67. (Currently amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) starting with an amino acid sequence of a portion of an insecticidal protein derived from *Bacillus thuringiensis* (*B.t.*), wherein a wild-type *B.t.* coding sequence from which the portion was derived includes ATTTA sequences and polyadenylation signal sequences listed in Table II; eoding sequences that encode portions of one or more insecticidal polypeptides derived from *Bacillus thuringiensis* (*B.t.*); and
- (b) making a structural gene that comprises a coding sequence that encodes the amino acid sequence and that contains fewer ATTTA sequences and fewer of said polyadenylation signal sequences than the wildtype *B.t.* coding sequence combining the coding sequences to form a structural gene that encodes an insecticidal protein,

wherein said coding sequences and the structural gene are devoid or substantially devoid of polyadenylation signal sequences listed in Table II.

- 68. (Currently amended) The method of claim 67, wherein said coding sequences and the structural gene are step (b) comprises making a structural gene that is devoid or substantially devoid of said ATTTA sequences and devoid or substantially devoid of said polyadenylation signal sequences.
- 69. (Currently Amended) The method according to any one of claims 47, 49-51, and 53-57 [[55]], wherein the structural gene made according to the method is more highly expressed in a dicot plant cell than a structural gene that comprises the starting coding sequence(s) of step (a).
- 70. (Currently Amended) The method according to any one of claims 59<sub>-</sub> 62 and 67, wherein the structural gene made according to the method is more highly expressed in a dicot plant cell than a structural gene that comprises the wild-type <u>B.t.</u> <u>Bacillus</u> gene sequence(s) encoding polypeptide(s) from which the amino acid sequence of the insecticidal protein is derived.

## 71.- 72. (Cancelled)

73. (Currently Amended) The method according to any one of claims 49<sub>-</sub>51, 53-55, 52, 56, 60-61, 64, and 68, wherein the structural gene made according to the method contains no ATTTA sequences.

Atty. Docket No.: 28079/41785

74-76. (Cancelled)

- 77. (Currently Amended) The method according to any one of claims <u>47</u>, <u>49</u>, <u>51</u>, [[48,]] 53, <u>55</u>, <u>59</u>, [[57,]] 61, <u>64</u>, and <u>68</u>, <del>63</del>, and <del>67</del>, wherein the structural gene made according to the method contains no polyadenylation signal sequences listed in Table II.
- 78. (Currently Amended) The method according to any one of claims 47, 49, 51, 55, 59-64, 63, and 67, wherein the structural gene made according to the method contains a (G+C) content of about 50%.
- 79. (Currently Amended) The method according to claim <u>47, 51, or 55,</u> [[81,]] wherein the starting coding sequence[[(s)]] of step (a) has <del>(have)</del> (A + T) content of about 62%.
- 80. (Currently Amended) The method according to claim <u>59 or 60</u>, [[84,]] wherein the wild-type *Bacillus* gene <u>sequence(s)</u> sequences from *B.t.* have an (A+ T) content of about 62%.
  - 81. (Cancelled)
- 82. (Currently Amended) The method according to any one of claims 47, 49, 51, and 53-55 and 67, wherein the starting coding sequence[[(s)]] of step (a) is [[are]] derived from a *Bacillus thuringiensis* (*B.t.*) crystal protein gene.
- 83. (Currently Amended) The method of any one of claims 47, <u>49</u>, 51, 55, and 67, wherein the starting coding sequence[[(s)]] of step (a) <u>is</u> [[are]] derived from a *Bacillus thuringiensis* (*B.t.*) P2 protein or a *B.t. entomocidus* gene.
  - 84. (Canceled)
- 85. (Currently Amended) The method according to any one of claims 59 and to 62, wherein the wild-type gene sequences sequence(s) comprise *Bacillus thuringiensis* (*B.t.*) crystal protein gene sequences.

86. (Currently Amended) The method according to any one of claims 59 to and 62, wherein the wild-type gene sequences sequences(s) comprise *Bacillus* thuringiensis (B.t.) P2 gene sequences or B.t. entomocidus gene sequences.

- 87. (Canceled)
- 88. (Currently Amended) The method according to any one of claims 47, 49-51, 53-54, 59-64, 63-and 67, wherein the insecticidal protein is a *Bacillus thuringiensis* (*B.t.*) crystal protein.
- 89. (Currently Amended) The method according to any of claims 47,  $\underline{49}$ , 51, 55 and 67, wherein the starting coding sequence[[(s)]] of step (a) are is derived from *B.t.* tenebrionus.
- 90. (Currently Amended) The method according to any one of claims 47, 49, 51, 59-60, and 63-64, wherein the insecticidal protein is a *B.t.* P2 protein or a *B.t.* entomocidus protein.
  - 91-92. (Cancelled)
- 93. (Currently Amended) The method according to any one of claims 47, 49, 51, and 55, and 67, wherein the coding sequence of step (a) comprises a sequence that encodes sequence(s) of step (a) encode(s) an insecticidal fragment of a <u>B.t.</u> Bacillus insecticidal protein.
- 94. (Currently Amended) The method according to any one of claims 47, 49, 51, and 55, and 67, wherein the coding sequence(s) of step (a) encode(s) a full length <u>B.t.</u> <u>Bacillus</u> insecticidal protein.
- 95. (Currently Amended) The method according to any one of claims 59<u>-</u>64 and 67 [[63]], wherein the insecticidal protein derived from <u>B.t.</u> Bacillus comprises an insecticidal fragment of a <u>B.t.</u> Bacillus insecticidal protein.
- 96. (Currently Amended) The method according to any one of claims 59<sub>-</sub>64 and 67 [[63]], wherein the insecticidal protein derived from <u>B.t. Bacillus</u> comprises a full length B.t. Bacillus insecticidal protein.
  - 97. (Canceled)

98. (Currently Amended) The method according to any one of claims 47, 49, 51, [[55,]] 59, 60, 63-64, and 67, wherein the insecticidal protein encoded by the structural gene comprises an amino acid sequence that is identical to the amino acid sequence of an insecticidal protein from *B.t. Bacillus*, or an insecticidal fragment thereof.

## 99. (Cancelled)

- 100. (Currently Amended) The method according to any one of claims 47, 49-51, [[55,]] 53-57, 59-64, and 67 [[63]], comprising avoiding the introduction of sense codons that are rarely found in plant genomes into the resultant structural gene.
- 101. (Currently Amended) The method according to any one of claims 47, 49-51, [[55,]] 53-57, 59-64, and 67 [[63]], comprising avoiding, in the resultant structural gene, the introduction of sense codons that contain a TA doublet.
- 102. (Currently Amended) The method according to any one of claims 47, 49-51, [[55,]] 53-57, 59-64, and 67 [[63]], comprising avoiding, in the resultant structural gene, the introduction of sense codons that contain a CG doublet.
- 103. (Currently Amended) The method according to any one of claims 47 and 55 claim 47 or 49, further comprising reducing the number of regions in the coding sequence(s) with greater than five consecutive adenine and thymine (A+T) nucleotides by substituting sense codons for codons in the coding sequence(s).
- 104. (Currently Amended) The method according to claim 51 or 55, further comprising reducing the number of regions in said portion with greater than five consecutive adenine and thymine (A+T) nucleotides by substituting sense codons for codons in the portion.
- 105. (Currently Amended) The method according to any one of claims 59, 60, 63-64, and 67, wherein the structural gene comprises a coding sequence that does not contain more than five consecutive adenine and thymine (A+T) nucleotides.
- 106. (Currently Amended) The method according to any one of claims 47 and 55 claim 47 or 49, further comprising truncating the coding sequence to yield a truncated structural gene that encodes a truncated protein that retains insecticidal activity.

107. (Currently Amended) The method according to any one of claims 47, 49-51, [[55,]] 53-57, 59-64, 63, and 67, further comprising attaching a plant promoter to the

structural gene.

- 108. (Currently Amended) The method according to any one of claims 47, 49-51, [[55,]] 53-57, 59-64, 63, and 67, further comprising including in the structural gene a sequence that encodes an amino-terminal chloroplast transit peptide or a secretory signal sequence.
- 109. (Currently Amended) The method according to any one of claims 47, 49-51, [[55,]] 53-57, 59-64, 63, and 67, further comprising attaching to the structural gene a 3' non-translated nucleotide sequence that comprises a plant polyadenylation signal.
- 110. (Currently Amended) The method according to any one of claims 47, 49, 51, and 55, wherein the making step comprises performing site directed mutagenesis on a coding sequence from *Bacillus thuringiensis* to make the structural gene.
- 111. (Currently Amended) The method according to any one of claims 47, 49, 51, and 55, wherein the making comprises *de novo* synthesis of a fully synthetic structural gene.
- 112. (Currently Amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) starting with a sequence derived from *Bacillus thuringiensis (B.t.)*, said sequence comprising: (i) a coding sequence for an insecticidal protein having an amino acid sequence, or (ii) an amino acid sequence of the insecticidal protein; and
- (b) making a structural gene that comprises a coding sequence that encodes the amino acid sequence and that is devoid or substantially devoid of ATTTA sequences, <u>and</u> [[or]] devoid or substantially devoid of polyadenylation signal sequences listed in Table II.
- 113. (Currently Amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) designing a nucleotide sequence that encodes an insecticidal protein derived from *Bacillus thuringiensis (B.t.)* and that contains a reduced number of ATTTA sequences or Table II polyadenylation signal sequences <u>listed in Table II</u>, compared to a wild

type <u>B.t.</u> <u>Bacillus</u> coding sequence from which the insecticidal protein was derived, <u>wherein</u> the number of said polyadenylation signal sequences is reduced compared to the wild type <u>B.t.</u> coding sequence by substituting codons while maintaining the encoded amino acids; and

- (b) making a structural gene that comprises the nucleotide sequence, that encodes the insecticidal protein, and that is characterized by the reduced number of ATTTA sequences or Table II said polyadenylation signal sequences, compared to the wild type <u>B.t.</u> <u>Bacillus</u> coding sequence.
- 114. (Currently Amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising: The method of claim 113
- (a) designing a nucleotide sequence that encodes an insecticidal protein derived from *Bacillus thuringiensis* (*B.t.*) and that contains a reduced number of ATTTA sequences or Table II polyadenylation signal sequences, compared to a wild type *B.t. Bacillus* coding sequence from which the insecticidal protein was derived, wherein the insecticidal protein comprises a protein selected from the group consisting of: (a) *B.t. Bacillus* thuringiensis (*B.t.*) insecticidal proteins; and (b) insecticidal fragments of (a); and insecticidal fusions derived from more than one *B.t.* insecticidal protein; and
- (b) making a structural gene that comprises the nucleotide sequence, that encodes the insecticidal protein, and that is characterized by the reduced number of ATTTA sequences or Table II polyadenylation signal sequences, compared to the wild type *Bacillus* eoding sequence.
- 115. (Currently Amended) The method of claim 113 or 114, wherein the wild-type <u>B.t. Bacillus</u> coding sequence(s) comprises a plurality of both said ATTTA and said polyadenylation signal ATTTA sequences, wherein the nucleotide sequence designed in step (a) contains a reduced number of the ATTTA sequences compared to the wild-type <u>B.t.</u> coding sequence, wherein the number of the ATTTA sequences is reduced by substituting codons while maintaining the encoded amino acids, and wherein the structural gene made according to step (b) contains fewer ATTTA sequences and fewer polyadenylation signalis characterized by the reduced number of ATTTA sequences compared to the wild-type <u>B.t.</u> <u>Bacillus</u> coding sequence(s).

116. (Currently Amended) The method according to <u>claim 113 or 114 any</u> one of claims 113-115, wherein the structural gene made according to the method is more highly expressed in a dicot plant cell than a structural gene that consists of <u>the</u> wild type <u>B.t.</u> <u>Bacillus</u> coding sequence(s) from which the insecticidal protein was derived.

- 117. (Currently Amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) designing a nucleotide sequence that encodes an insecticidal protein derived from *Bacillus thuringiensis* (*B.t.*) and that is devoid or substantially devoid of ATTTA sequences, or devoid or substantially devoid of Table II polyadenylation signal sequences listed in Table II; and
- (b) making a structural gene that comprises the nucleotide sequence and that encodes the insecticidal protein, wherein the structural gene is devoid or substantially devoid of ATTTA sequences, or devoid or substantially devoid of said Table II polyadenylation signal sequences.
  - 118. (Currently Amended) The method of claim 117,

wherein step (a) further comprises designing the nucleotide sequence to be devoid or substantially devoid of ATTTA sequences, and

wherein the structural gene <u>made according to step (b)</u> is devoid or substantially devoid of the ATTTA sequences, and devoid or substantially devoid of the polyadenylation signal sequences.

- 119. (Currently Amended) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) starting with a coding sequence, derived from *Bacillus thuringiensis* (*B.t.*), that contains a plurality of sequences selected from the group consisting of ATTTA sequences and polyadenylation signal sequences listed in Table II [[I]], and that encodes an insecticidal protein having an amino acid sequence;
  - (b) making a structural gene that comprises a coding sequence that:
    - (i) encodes the amino acid sequence of the insecticidal protein; and

(ii) contains fewer polyadenylation signal sequences listed in Table II or fewer ATTTA sequences, compared to the corresponding coding sequence derived from *B.t. Bacillus*.

- 120. (Currently Amended) A method of making a structural gene that encodes a protein, the method comprising:
- (a) starting with a coding sequence that encodes a <u>protein</u> <del>polypeptide</del> and that contains a <del>plurality of sequences selected from the group consisting of ATTTA</del> <del>sequences and polyadenylation signal sequences listed in Table II;</del>
- (b) reducing the number of said ATTTA sequences or the number of said polyadenylation signal sequences in the coding sequence by substituting sense codons for codons in the coding sequence; and
- (c) making a structural gene that comprises a coding sequence that includes the codons substituted according to step (b) and is characterized by the reduced number of ATTTA sequences or Table II polyadenylation signal sequences, and that encodes the [[a]] protein.
- 121. (Currently Amended) The method of claim 120, wherein the starting coding sequence of step (a) contains ATTTA sequences, and wherein step (b) further comprises reducing the number of said ATTTA sequences and the number of said polyadenylation signal sequences in the coding sequence by substituting sense codons for codons in the coding sequence.
- 122. (Currently amended) A method of making a structural gene that encodes a protein, the method comprising:
- (a) starting with a coding sequence that encodes <u>an amino acid sequence</u> <del>a</del> <del>polypeptide</del> and that contains <del>a plurality of sequences selected from the group consisting of ATTTA sequences and</del> polyadenylation signal sequences listed in Table II;
- (b) reducing the number of said ATTTA sequences or the number of said polyadenylation signal sequences in a portion of the coding sequence by substituting sense codons for codons in said portion, while maintaining the amino acid sequence; and

Application No. 08/434,105 Atty. Docket No.: 28079/41785

Reply to Office Action of 2008 November 25

(c) making a structural gene that comprises said portion with the substitute codons and the reduced number of ATTTA or polyadenylation signal sequences; and

- (d) making a DNA construct that comprises the structural gene and at least one sequence selected from the group consisting of a plant promoter or a plant virus promoter, wherein the structural gene comprises a nucleotide sequence that encodes a protein.
- 123. (Currently Amended) The method of claim 122, wherein the starting coding sequence contains ATTTA sequences, and wherein step (b) further comprises reducing the number of said ATTTA sequences and the number of said polyadenylation signal sequences in said portion, while maintaining the amino acid sequence.
- 124. (Currently Amended) A method of making a structural gene that encodes a protein, the method comprising:
- (a) starting with coding sequences, from one or more structural genes that encode a <u>protein</u> polypeptide and that contain a plurality of sequences selected from the group consisting of ATTTA sequences and polyadenylation signal sequences listed in Table II;
- (b) reducing the number of said ATTTA sequences or the number of said polyadenylation signal sequences in the coding sequences by substituting sense codons for codons in the coding sequences; and
- (c) making a structural gene that comprises the coding sequences with the codons substituted according to step (b) and characterized by the reduced number of ATTTA or polyadenylation signal sequences, wherein the structural gene comprises a nucleotide sequence that encodes the [[a]] protein.
- 125. (Currently Amended) The method of claim 124, wherein the starting coding sequences of step (a) contain ATTTA sequences, and wherein step (b) further comprises reducing the number of said ATTTA sequences and the number of said polyadenylation signal sequences in said coding sequences by substituting sense codons for codons in the coding sequence.
- 126. (Currently Amended) A method of making a structural gene that encodes a protein, the method comprising:

(a) starting with an amino acid sequence of <u>a</u> an insecticidal protein, wherein the amino acid sequence is derived from a coding sequence that contains polyadenylation signal sequences listed in Table II and ATTTA sequences; [[and]]

- (b) making a structural gene that comprises a coding sequence that encodes the amino acid sequence and that is devoid or substantially devoid of ATTTA sequences, and [[or]] devoid or substantially devoid of polyadenylation signal sequences listed in Table II; and
- (c) making a DNA construct that comprises the structural gene and at least one sequence selected from the group consisting of a plant promoter or a plant virus promoter.
- 127. (Currently Amended) The method of claim 119 [[126]], wherein the coding sequence derived from *B.t.* contains ATTTA sequences, and wherein step (b) comprises making a structural gene that comprises a coding sequence that is devoid or substantially devoid contains fewer of the ATTTA sequences and devoid or substantially devoid of the polyadenylation signal sequences compared to the corresponding coding sequence derived from *B.t.*
- 128. (Currently Amended) A method of making a structural gene that encodes a protein, the method comprising: combining coding sequences to form a structural gene that encodes an insecticidal protein derived from *Bacillus thuringiensis* (*B.t.*), wherein said coding sequences and the structural gene are devoid or substantially devoid of polyadenylation signal sequences listed in Table II.
- 129. (Previously Presented) The method of claim 128, wherein said coding sequences and the structural gene are devoid or substantially devoid of ATTTA sequences.
- 130. (Currently Amended) The method according to any one of claims <u>112-115</u>, and <u>117[[120]]-129</u>, further comprising attaching a plant promoter to the structural gene.
- 131. (Currently Amended) The method according to any one of claims <u>112-115</u>, and <u>117[[120]]-129</u>, further comprising including in the structural gene a sequence that encodes an amino-terminal chloroplast transit peptide or a secretory signal sequence.

132. (Currently Amended) The method according to any one of claims <u>112-115</u>, and <u>117</u>[[120]]-129, further comprising attaching to the structural gene a 3' non-translated nucleotide sequence that comprises a plant polyadenylation signal.

- 133. (Currently Amended) The method according to any one of claims 112, 119-125, and 127120 125, wherein the structural gene made according to the method is more highly expressed in a dicot plant cell than a structural gene that comprises the starting coding sequence(s) of step (a).
- 134. (Currently Amended) The method according to any one of claims <u>112-115</u>, and <u>117[[120]]-129</u>, wherein the structural gene made according to the method contains no ATTTA sequences.
- 135. (Currently Amended) The method according to any one of claims <u>112-115</u>, and <u>117[[120]]-129</u>, wherein the structural gene made according to the method contains no polyadenylation signal sequences listed in Table II.
- 136. (Currently Amended) The method according to any one of claims <u>112</u>, <u>119-125</u>, and <u>127</u><del>120</del> <u>125</u>, wherein the starting coding sequence(s) of step (a) is (are) derived from *Bacillus thuringiensis* and has (have) an (A + T) content of about 62%.
- 137. (Currently Amended) The method according to any one of claims <u>112-115</u> and <u>117-129120-127</u>, comprising avoiding the introduction of sense codons that are rarely found in plant genomes into the resultant structural gene.
- 138. (Currently Amended) The method according to any one of claims <u>112-115</u> and <u>117-129</u> 120-127, comprising avoiding, in the resultant structural gene, the introduction of sense codons that contain a TA doublet.
- 139. (Currently Amended) The method according to any one of claims <u>112-115</u> and <u>117-129120-127</u>, comprising avoiding, in the resultant structural gene, the introduction of sense codons that contain a CG doublet.
- 140. (Currently Amended) The method according to any one of claims 112, 119-125, and 127120 125, further comprising reducing the number of regions in the coding sequence(s) with greater than five consecutive adenine and thymine (A+T) nucleotides by substituting sense codons for codons in the coding sequence(s).

141. (Currently Amended) The method according to any one of claims <u>112-115</u>, and <u>117[[120]]-129</u>, wherein the structural gene comprises a coding sequence that does not contain more than five consecutive adenine and thymine (A+T) nucleotides.

- 142. (New) The method according to any one of claims 47, 49, 51, 55, 59, 60, 63-64, 112-115, and 117-129, further comprising including in the structural gene a sequence that encodes a secretory signal sequence.
- 143. (New) The method of claim 115, wherein the structural gene is devoid or substantially devoid of the ATTTA sequences, or devoid or substantially devoid of the polyadenylation signal sequences.
- 144. (New) A method of making a DNA construct for expression of an insecticidal protein derived from *Bacillus thuringiensis* (*B.t.*), the method comprising combining a 5' nontranslated sequence, a structural gene, and a 3' nontranslated region to form a construct for expression of the insecticidal protein,

wherein the 5' nontranslated sequence contains a plant promoter or a plant virus promoter,

wherein the structural gene comprises a sequence that encodes the insecticidal protein derived from *B.t.* and is devoid or substantially devoid of polyadenylation signal sequences listed in Table II, and

wherein the 3' nontranslated sequence comprises a polyadenylation signal.

- 145. (New) A method of making a structural gene that encodes an insecticidal protein, the method comprising:
- (a) starting with a sequence derived from *Bacillus thuringiensis* (*B.t.*), said sequence comprising: (i) a coding sequence for an insecticidal protein having an amino acid sequence, or (ii) an amino acid sequence of the insecticidal protein; and
- (b) making a structural gene that comprises a coding sequence that encodes the amino acid sequence and that is devoid or substantially devoid of polyadenylation signal sequences listed in Table II.
- 146. (New) A method of making a structural gene that encodes a protein, the method comprising:

Reply to Office Action of 2008 November 25

(a) starting with an amino acid sequence of a protein, wherein the amino acid sequence is derived from a coding sequence that contains polyadenylation signal sequences listed in Table II;

- (b) making a structural gene that comprises a coding sequence that encodes the amino acid sequence and that is devoid or substantially devoid of polyadenylation signal sequences listed in Table II; and
- (c) making a DNA construct that comprises the structural gene and at least one sequence selected from the group consisting of a plant promoter or a plant virus promoter.
- 147. (New) The method according to any one of claims 47, 49-51, 53-57, 59-68, 112-115, 117-121, 124-125, 127-129, 143, and 145-146, further comprising making a DNA construct that comprises the structural gene and at least one sequence selected from the group consisting of a plant promoter or a plant virus promoter.
- an insecticidal protein derived from *Bacillus thuringiensis* (*B.t.*) and that involves starting with a wild type *B.t.* coding sequence or with an amino acid sequence encoded thereby, the improvement which comprises reducing the number of polyadenylation signal sequences listed in Table II when making the structural gene, compared to the number of said polyadenylation signal sequences in the wild type *B.t.* coding sequence, by substituting codons relative to the wild-type *B.t.* coding sequence while maintaining the amino acid sequence.
- 149. (New) In the method of claim 148, the further improvement which comprises reducing the number of ATTTA sequences when making the structural gene, compared to the number of said ATTTA sequences in the wild type *B.t.* coding sequence, by substituting codons relative to the wild type *B.t.* coding sequence while maintaining the amino acid sequence.
- 150. (New) In the method of claim 148 or 149, the further improvement which comprises reducing the number of occurrences of greater than five consecutive adenine and thymine (A + T) nucleotides, compared to the wild type B.t. coding sequence, by

substituting codons relative to the wild type B.t. coding sequence while maintaining the amino acid sequence.

- 151. (New) In the method of claim 148, the further improvement wherein the structural gene made according to the method is devoid or substantially devoid of said polyadenylation signal sequences.
- 152. (New) In the method of claim 149, the further improvement wherein the structural gene made according to the method is devoid or substantially devoid of ATTTA sequences.
- 153. (New) In the method of claim 148, the improvement wherein the structural gene made according to the method contains none of said polyadenylation signal sequences.
- 154. (New) In the method of claim 149, the improvement wherein the structural gene made according to the method contains no ATTTA sequences.
- 155. (New) In the method of claim 148 or 149, the further improvement wherein the insecticidal protein is a *B.t.* crystal protein.
- 156. (New) In the method of claim 148 or 149, the further improvement wherein the wild type *B.t.* coding sequence comprises a sequence that encodes an insecticidal fragment of a *B.t.* insecticidal protein.
- 157. (New) In the method of claim 148 or 149, the further improvement which comprises avoiding, in the resultant structural gene, the introduction of codons that contain a TA doublet.
- 158. (New) In the method of claim 148 or 149, the further improvement which comprises avoiding, in the resultant structural gene, the introduction of codons that contain a CG doublet.
- 159. (New) In the method of claim 148 or 149, the further improvement which comprises attaching a plant promoter to the structural gene.
- 160. (New) In the method of claim 148 or 149, the further improvement which comprises including in the structural gene a sequence that encodes an amino-terminal chloroplast transit peptide.

161.

Reply to Office Action of 2008 November 25

sequence.

162. (New) In the method of claim 148 or 149, the further improvement

which comprises including in the structural gene a sequence that encodes a secretory signal

which comprises attaching to the structural gene a 3' non-translated nucleotide sequence that

comprises a plant polyadenylation signal.

163. (New) In the method according to any one of claims 148, 149, 151,

(New) In the method of claim 148 or 149, the further improvement

Atty. Docket No.: 28079/41785

and 152, the further improvement that comprises making a DNA construct that comprises the

structural gene and at least one sequence selected from the group consisting of a plant

promoter or a plant virus promoter.

20